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The Lethal Doses of CO_2, N_2, NH_3 and H_2S for Soil Arthropoda

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With 5 figures in the text

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1 Introduction

The gaseous phase of the soil is continously changing, due to natural causes, such as the changes in the seasons throughout the year, the texture and structure of the soil itself, temperature, humidity, rain, geographical position, depth of the soil and biological activities; or caused by the use of manures, alternation of various plants, crops and tillage (Baver, 1948).

According to Russel and Appleyard (1915), air in the soil has a CO_2 content about six to seven times higher than the atmosphere, the N_2 content is slightly higher, while the quantity of NH_3 in the soil is in proportion to the decomposition of organic matter. H_2S occurs, according to Giesecke (1930), in small amounts in the soil air and increases in its percentage are due to the rotting processes which occur in the soil.

An important common factor which causes rapid changes in the concentrations of these four gases, in one and the same soil and at the same time, is the use of manure. The microbiological activities will increase, and thus increase the concentrations of these gases (Russel and Appleyard 1915).

In this field, the resistance of soil animals to gases, the work of Kupka and Schaerfenberg (1947) working, with 100% CO_2 on large size soil animals can be cited here. No other work in connection with this investigation could be found on other soil animals.

I express my indebtedness to Prof. Dr. W. Kühnelt, Head of the Department of Zoology, at the University of Vienna, for suggesting to me this research, which was carried out in his department.

2 Experiments and results

The small athropods used were collected from the neighbourhood of Vienna, from three differently aerated media, namely a normal soil (a forest soil), a compost (a vegetable manure) and a natural manure (feces of oxen).

The following species were selected from the normal soil: an isopod (*Porcellio scaber* Latr. 1814); a dipterous larva (*Pachygaster* sp.); from the Acari, four Oribatei aptyctima (*Damaeus onustus* C. L. Koch 1841 = *Belba geniculosa* Oudmans 1929 ; *Carabodes femoralis* Nic. 1855; *Achipteria coleoptrata* L. 1758; *Xenillus tegeocranus* Herm. 1804) two Oribatei ptyctima (*Tropacarus pulcherrimus* Berlese 1887; *Phthiracarus ligneus* Willm. 1931) a Gamasid (*Holoparasitus calcaratus* C. L. Koch 1840) and a Collembola (*Onychiurus granulosus* Stach 1930). From the compost two species, a Gamasid (*Macrocheles merdarius* Berlese 1889) and a Collembola (*Hypogastrura bengtssoni* Ågren 1904). From the natural manure two other species, a Gamasid (*Macrocheles muscae domesticae* Scopoli 1792) and a Collembola (*Isotomina thermophila* Axelson 1900).

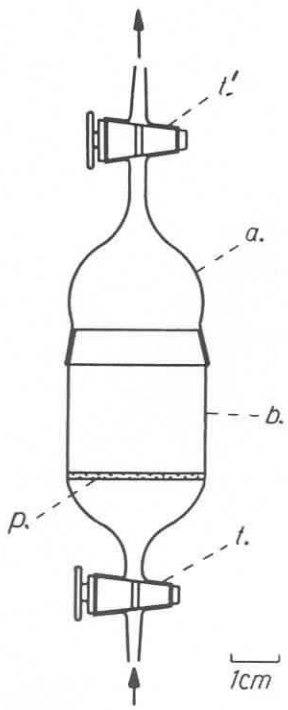

Fig. 1. Animal chamber, a) upper part, b) lower part, t) upper tap, t') lower tap, p) porous plate.

The experiments were carried on in a glass animal chamber (Fig. 1), which could be tightly closed, the gas being transfered from the gascylinder to an aspiractor, then conducted to the animal chamber. The gas current passes from the lower tap (t) through a porous plate (p). which distributes it uniformly in the whole chamber, and leaves through the upper tap (t'). By closing the two taps the atmosphere in the chamber remains constant throughout the whole ex periment.

In order to find out the lethal doses (LD 50, the concentration at which 50% of the number of individuals of a species died in a certain period), experiments over a period of seven days were performed with various concentrations of the gases, starting with high ones, having a fatal effect (100% death) and then with decreasing concentrations, until those in which the animals were resistant (5—10% death). The LD 50 lies between these maximal and minimal doses.

In every experiment the atmosphere in the animal chamber was controlled for the seven days.

The LD 50 values for the previously mentioned species are tabulated as follows (Table 1):

Table 1
Concentration of different gases at which 50% of the number of individuals of a species died in a certain period (LD 50)

	CO_2%	N_2%	NH_3%	H_2S%
Damaeus onustus	9,1	100	0,0023	0,8
Carabodes femoralis	7,9	100	0,0023	0,75
Achipteria coleoptrata	7,5	98	0,0023	0,68
Xenillus tegeocranus	6,7	96,8	0,0022	0,66
Tropacarus pulcherrimus	5,3	97,2	0,0014	0,09
Phthiracarus ligneus	1,9	96	0,00056	0,06
Holoparasitus calcaratus	5,3	93,7	0,00062	0,08
Macrocheles merdarius	11,8	93,7	0,0065	0,7
Macrocheles muscae domesticae	23	—	0,0095	0,7
Onychiurus granulosus	1,7	96,2	0,00032	0,05
Hypogastrura bengtssoni	6,7	96,4	0,001	2,3
Isotomina thermophila	17	96,9	0,003	1,3
Pachygaster sp.	30	—	0,005	6
Porcellio scaber	1,2	88,4	0,00045	0,06

3 Discussion of the results

The four *Oribatai aptyctima* were more resistant than the two *ptyctima* species, with one exception in the case of N_2, where *Tropacarus pulcherrimus* was more resistant than *Xenillus tegeocranus*.

In CO_2 there is a gradation in resistance between the four *aptyctima* species, ranging from *Damaeus onustus* as the most resistant, through *Carabodes femoralis*, *Achipteria coleoptrata*

and finally *Xenillus tegeocranus*. This gradation is also present with the other gases, except NH_3 where the first three were equally and more resistant than the fourth species *Xenillus tegeocranus*.

Variations in resistance to CO_2 between the two groups Gamasina and Collembola living in the three differently aerated media, namely normal soil, compost and natural manure also exhibite peculiar results. The species living in compost occupy a middle position between those of the normal soil, on the one hand, having the least resistance, and those of manure on the other, having the highest resistance. The LD 50 values for *Macrocheles muscae domesticae* attains 23% and for *Isotomina thermophila* 17% (Fig. 2).

This middle position disappears in the experiments with N_2, where the gamasids *Macrocheles merdarius* have the same lethal dose as those of the normal soil, *Holoparasitus calcaratus*. Those of manure, *Macrocheles muscae domesticae* are higher, whilst with the Collembola the difference beween the three species does not exceed 0,7%. (Fig. 3).

The middle position of the compost species reappears once more in the experiments with NH_3 (Fig. 4), whilst in H_2S the Collembola from compost, *Hypogastrura bengtssoni*, have the highest resisteance in comparison with those of the normal soil and manure; those of manure *Isotomina thermophila* being now in a middle position. The gamasids from compost were equally resistant to those of manure (Fig. 5).

It is assumed here that a compost formed from organic matter obtained from plant debris only, may be a source of more H_2S, while a natural manure, faeces of oxen, will contain more NH_3. This might explain the variations in resistance between these species. Those living in compost were more resistant to H_2S, while those living in a manure withstood better the effect of NH_3.

At the same time, these four species withstood higher concentrations of CO_2 better than those of the normal soil, since in both samples, compost and manure, the percentage of CO_2 according to Russel and Appleyard (1915) is higher. While in an anaerobic atmosphere, i. e. N_2, the six species, of Gamasina and Collembola, did not exhibit such great differences in resitance.

The LD 50 of *Pachygaster* sp. shows the highest values in comparison to the other species in three gases, namely CO_2, N_2 and H_2S, while in NH_3 they follow in their resistance the gamasid from manure, *Macrocheles muscae domesticae*. The biology of this insect gives the reasons for its high resistance. They are eurytopic, widely distributed, the female lays its eggs in normal soil as well as in manure and compost, it requires 2—3 years for maturation. This is in addition to their great ability to control their outer respiratory openings, espe cially in CO_2.

The isopods *Porcellio scaber* were extremely susceptible, their LD 50 values are the lowest among the species discussed above except in the cases of NH_3 and H_2S where they were more resistant than the Collembolan, *Onychiurus granulosus*, from the normal soil. Here it could be said that the physiological mechanism withstood the effect of these poisonous gases better than the mechanism functioning in anaerobiosis, in comparison to *Onychiurus granulosus*. Otherwiese the extremely low resistance of these gill-breathing land isopods, especially to CO_2 and N_2, is due, to the high permeability of their integument, in comparison to the other species. According to Edney and Spencer (1953), diffusion of CO_2 continues to take place through the integument, after blocking of the pleopods of *Porcellio scaber*. In Collembola, Ruppel (1953) reported that only $^2/_3$ of the total respiration takes place through the integument, while $^1/_3$ is regulated through the ventral tube. Such a diffusion will not play any role in the more resistant oribatids, where the integument, according to Vitzthum (1941) is so impermeable that it allows no skin respiration, permitting to resist wide variations of humidity.

In the experiments with N_2, the LD 50 is not attained in 100% N_2 in the cases of *Macrocheles muscae domesticae* and *Pachygaster* sp. since more than 50% of them lived in this

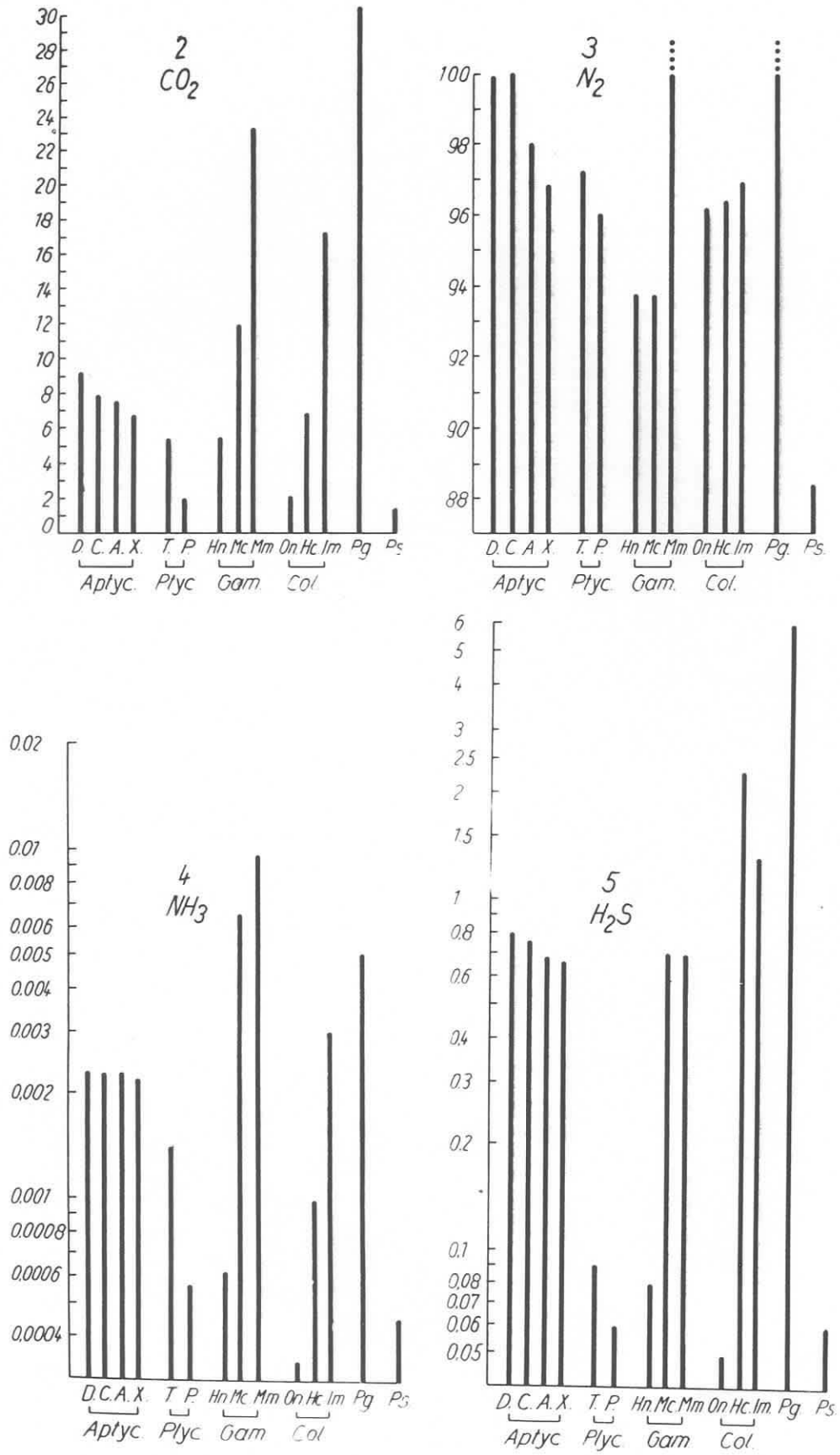
2
CO_2
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
D. C. A. X.
T. P.
Hn Mc Mm
On Hc Im
Pg
Ps
Aptyc.
Ptyc
Gam.
Col.
3
N_2
100
98
96
94
92
90
88
4
NH_3
0.02
0.01
0.008
0.006
0.005
0.004
0.003
0,002
0.001
0.0008
0.0006
0.0004
5
H_2S
6
5
4
3
2.5
2
1.5
1
0.8
0.6
0.4
0.3
0.2
0.1
0.08
0.07
0.06
0.05

Fig. 2—5

atmosphere for seven days. In other cases, as in *Damaeus onustus* and *Carabodes femoralis*, the LD 50 is 100% N_2. In most of the other species, the values are not far below 100% (86 to 98%) N_2.

In the case of CO_2 the LD 50 ran from 30% for the *Pachygaster larva*, 23% for *Macrocheles muscae domesticae* and 17% for *Isotomina thermophila*, which are the highest values; while in other cases such as *Phthiracarus ligneus*, *Onychiurus granulosus and Porcellio scaber* they do not exceed 1,9%. The LD 50 of the other species lies between these values.

On the case of NH_3 the differences in the LD 50 are great, attaining an order of magnitude as in the cases between the gamasids *Macrocheles muscae domesticae* from manure (0,0095) and *Holoparasitus calcaratus* from normal soil (0,00062). All the mentioned LD 50 values of NH_3 in the above table are far higher than the quantities of NH_3 normally present in nature (0,000082/100 l. soil air; FODOR, 1875).

The relationship between the LD 50 values with H_2S covers the same order as NH_3, except in two cases, namely the *Pachygaster sp.* (6%), which is the highest in comparison to the other species, followed by the Collembola from compost *Hypogastrura bengtssoni* (2,3%).

Both NH_3 and H_2S should be considered as highly poisonous gases. Their LD 50 values being extremely low in comparison to those of CO_2 and N_2.

From the above table it is evident that NH_3 is the most toxic among the four gases, the LD 50 values being the lowest; followed by H_2S and then CO_2. Nitrogen should be treated separately, since is has no poisonous effect on the animals. Some of them survived in a pure atmosphere of this gas for seven days, namely the species *Damaeus onustus*, *Carabodes femoralis*, *Macrocheles muscae domesticae* and *Pachygaster sp.* These animals respire anaerobically and will live in this atmosphere as long as their body reserves permit.

Carbon dioxyd does not provide the animals with an anaerobic non-poisonous atmosphere as is the case with N_2, since an atmosphere composed of 50% CO_2 and 50% air has the same effect as 100% CO_2, i. e. a paralysing effect, the lethal doses for the present species start with 30% (*Pachygaster sp.*). Therfore its harmfull effect is not due to lack of oxygen.

HAZELHOFF (1927) said that the external respiratory apertures remain open in higher concentrations of CO_2, this will cause loss of water (evaporation) a process which will lead to dessication of the animal. However I do not agree with this dessication hypothesis, as a single causative factor, since when the surrounding atmosphere was kept moist, CO_2 had the same effect. This effect also occurs in aquatic animals where the factor of evaporation is totally eliminated.

BEADLE and BEADLE (1949) experimenting on *Sialis* and *Corethra*, found that it has no effect on the salt-absorbing mechanism, and its effect on the pH of the blood was not serious. Further researches were proposed by them on the neuro-muscular system.

Fig. 2. LD 50 of CO_2 Fig. 3. LD 50 of N_2
Fig. 4. LD 50 of NH_3 Fig. 5. LD 50 of H_2S

These LD 50 values give the variations in resistance between the above species. They are represented diagrammatically in the following figures (Figs. 2—5).

Ordinate: LD 50
Aptyc. Aptyctima
Ptyc. Ptyctima
Gam. Gamasina
Col. Collembola
D. *Damaeus onustus*
C. *Carabodes femoralis*
A. *Achipteria coleoptrata*
X. *Xenillus tegeocranus*
T. *Tropacarus pulcherrimus*

Abscissa: species
P. *Phthiracarus ligneus*
Hn. *Holoparasitus calcaratus* (normal soil)
Mc. *Macrocheles merdarius* (compost)
Mn. *Macrocheles muscae domesticae* (manure)
On. *Onychiurus granulosus* (normal soil)
Hc. *Hypogastrura bengtssoni* (compost)
Im. *Isotomina thermophila* (manure)
Pg. *Pachygaster larva*
Ps. *Porcellio scaber*

Concerning the variations in the degsees of toxicity between NH_3 and H_2S, BLAZKA (1958) reported that H_2S penetrates the tissues and diffuses out again after stopping the treatment. He further added that H_2S combines with cytochrome oxidases (the terminal member of the oxidising enzymes) which are not engaged in anaerobic metabolism, and the blood circulation is not interrupted. This finding provides here an explanation for the less complicated effect of H_2S during anoxia. in comparison to NH_3.

4 Summary

The influence of different gases (N_2, CO_2, H_2S and NH_3) within the atmosphere of the soil has been studied in some soil animals, especially mites and Collembola form three different habitats: normal soil, plant compost and manure (cow dung). The lethal dosis (LD 50) is in accordance with the normal occurrence of these gasese within the surroundings of the animals concerned. In general N_2 does not show any poisonous effects; even if an atmosphere of 100 % N_2 was applied. N_2 acts solely as an oxygen free atmosphere. CO_2 has a specific effect and paralyzes the animals even in mixtures 1:1 with normal air. H_2S is highly toxic but animals normally living in plant compost resist to 2—6 % H_2S for seven days. NH_3 proves to be the most poisonous substance studied. The highest resistence toward NH_3 has been shown for animals wich live in manure (cow dung). This is in accordance with the fact that H_2S is more likely to occur in compost and NH_3 in manure. The values are tabulated on page 3.

4 Zusammenfassung

Der Einfluß eines Zusatzes verschiedener Gase (Stickstoff, Kohlendioxyd, Schwefelwasserstoff und Ammoniak) zur Bodenluft wurde bei verschiedenen Bodentieren, vorwiegend Milben und Collembolen, untersucht. Die Tiere stammten aus drei verschiedenen Substraten: normaler Boden, Pflanzenkompost und aus einem Düngerstapel (Rindermist). Durch längere Einwirkung (bis zu 7 Tagen) von Gas-Luftgemischen bekannter Zusammensetzung auf die Tiere wurde die Letaldosis (LD 50) bestimmt, also derjenige Wert, bei dem 50 % der Tiere überleben. Stickstoff erwies sich als nicht giftiges sauerstofffreies Medium, während Kohlendioxyd auch bei Anwesenheit genügender Sauerstoffmengen eine spezifische Giftwirkung entfaltet, indem es die Tiere lähmt. Schwefelwasserstoff ist stark giftig, wird aber von den Bewohnern des Pflanzenkompostes noch in Konzentrationen von 2—6 % eine Woche lang ertragen. Alle anderen Tiere zeigen geringere Resistenz. Ammoniak zeigt die stärksten Giftwirkungen indem die resistentesten Tiere, sogar Bewohner des Düngers, weniger als ein hundertstel Prozent ertragen. Dieses Ergebnis ist einerseits deswegen interessant, weil im Pflanzenkompost eher H_2S, im Dünger eher NH_3 auftritt und die dort wohnenden Tiere dieser Situation angepaßt sind, andererseits deswegen, weil gezeigt wird, daß Ammoniak schon in geringer Konzentration alle Bodentiere schwer schädigt.

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